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## Research Note

### Effects of neonatal monocular enucleation on the number of GAD-positive puncta in rat visual cortex

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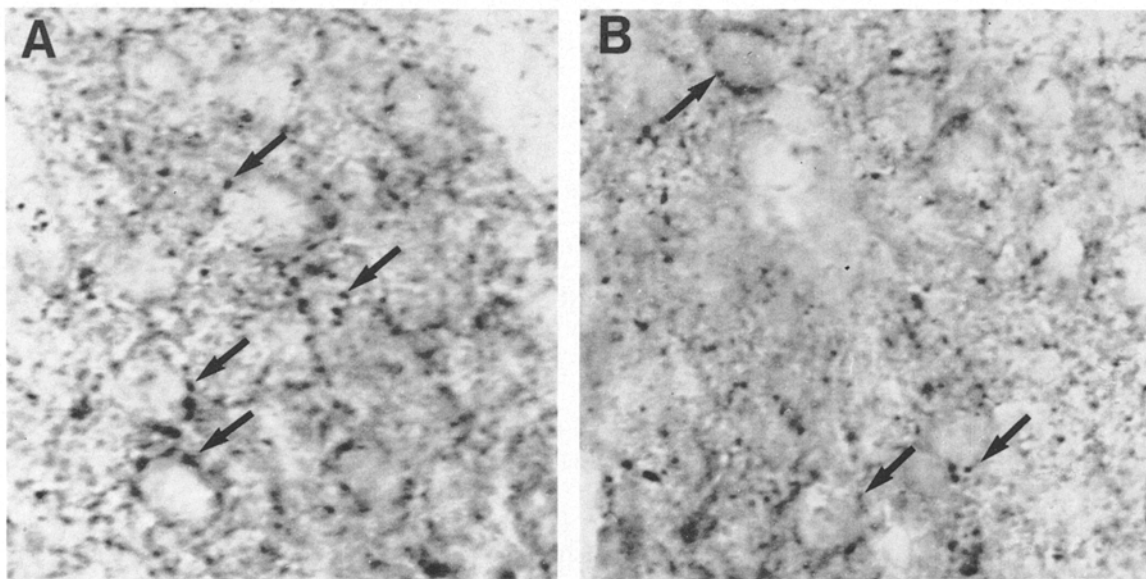
**Summary.** Rats that had one eye removed on the day of birth were examined at various postnatal ages with immunocytochemical methods to determine the effect on the development of the GABAergic axonal plexus in the visual cortex. The monocular segment of visual cortex contralateral to the enucleated orbit had 20–30% fewer GABAergic axon terminals than the monocular segment of visual cortex contralateral to the normal eye. Other cortical areas did not show any significant changes. These findings suggest that sensory deprivation of the visual cortex interferes with the normal development of GABAergic neurons.

**Key words:** GABAergic terminals – Visual cortex – Rat – Monocular enucleation

Previous studies have utilized immunocytochemical methods to demonstrate the somata and axon terminals of GABA and GAD-containing neurons in the visual cortex of mammals (Ribak 1978; Hendrickson et al. 1981; Somogyi and Hodgson 1985). These studies showed that aspiny and sparsely-spiny stellate neurons display GABA and GAD immunoreactivities and are likely to be GABAergic. Both basket and chandelier cells are included in the category of putative GABAergic neurons and the terminals of these cells have been shown to form symmetric synapses. These studies along with physiological studies (Creutzfeldt and Ito 1968; Creutzfeldt et al. 1974; Sillito 1977) have indicated that the GABAergic neurons in the visual cortex mediate inhibition and are involved in the processing of visual information.

Fifková (1970a) has analyzed the effect of monocular deprivation on the synaptic contacts of the visual cortex. The results of that study showed a 20% loss of synapses in the visual cortex supplied by the deprived eye. However, only synapses in the neuropil and those on the trunks of apical dendrites were analyzed whereas the axosomatic contacts were omitted from the analysis because their number was not large enough to calculate a density (Fifková 1970b). Since most GABAergic synapses are found associated with somata, axon initial segments and primary dendrites (Ribak 1978), they were probably not evaluated in these quantitative studies. Therefore, we have conducted a study to determine the effects of monocular enucleation on the GAD-containing axon terminals in visual cortex.

Laboratory-born, Long-Evans hooded rats that had one eye removed on the day of birth were sacrificed by intracardiac perfusions of aldehyde solutions at ages 18 to 90 days. Frozen sections (40  $\mu$ m) through the visual cortex were processed for immunocytochemistry to reveal the immunoreactive structures for glutamate decarboxylase (GAD), the synthesizing enzyme for GABA. Sections were processed as described previously using a sheep anti-GAD serum (Oertel et al. 1981). Since previous studies have indicated that GAD-positive punctate structures visible with the light microscope are identified as axon terminals with the electron microscope (Ribak 1978; Hendrickson et al. 1981), GAD-positive puncta were counted in the visual cortex of both hemispheres. Since synaptic transmission for cortical GABAergic neurons is considered to take place at axon terminals, a determination of the number of these structures is more functionally important than that of GABAergic cell bodies. A grid reticule was used to count GAD-positive puncta in 50  $\mu$ m X 100  $\mu$ m areas from the pial surface to the white matter. Counts were made without knowledge of the experi-



**Fig. 1A and B.** Photomicrographs of primary visual cortex that was incubated with GAD antibody to demonstrate the GAD-positive puncta (arrows) in a 60 day old rat. **A** shows the monocular segment of area 17 contralateral to the intact eye. **B** shows a similar region obtained contralateral to the enucleated orbit. The number of GAD-positive puncta appears to be reduced by about 20% as compared to **A**.  $\times 1050$

mental status of the animal or hemisphere under study. In addition, a number of control rats with intact eyes were included in this quantitative analysis. Differences in the number of GAD-positive puncta between the two hemispheres were analyzed with the Student's *t* test.

GAD-positive punctate structures were found in all layers of visual cortex as described previously (Ribak 1978). Somata were rarely found because these preparations were not pretreated with colchicine (Ribak et al. 1978). A comparison of visual cortex from the two hemispheres of experimental rats showed a decrease in the number of GAD-positive puncta in the monocular portion of area 17 contralateral to the removed eye (Fig. 1). A quantitative analysis of material from an animal sacrificed at 18 days of age showed that the monocular segment of area 17 contralateral to the enucleated eye had 33.8% fewer puncta than the monocular segment of area 17 contralateral to the normal eye ( $\bar{X}_{\text{contralateral to normal eye}} = 80.9$ ,  $SD = 14.9$ ;  $\bar{X}_{\text{contralateral to enucleation}} = 53.5$ ,  $SD = 11.5$ ;  $df = 21$ ;  $t = 5.31$ ,  $p < 0.01$ ). This quantitative difference in the number of puncta was slightly less in the older rats than in the younger rats (Fig. 2). The animal sacrificed at 60 days of age showed a 21.7% loss of GAD-positive puncta ( $t = 4.96$ ,  $p < 0.01$ ) and the one at 90 days of age showed a 19.1% loss ( $t = 4.90$ ,  $p < 0.01$ ). Other cortical areas (including areas 18a and 29d) did not display any significant differences in

the number of GAD-positive puncta between the hemispheres. In addition, the number of GAD-positive puncta in visual cortex from normal rats was similar to the number found in the visual cortex contralateral to the intact eye of monocularly enucleated rats.

The present results indicate a 20% loss of GABAergic axon terminals in the visual cortex contralateral to enucleation. These data are similar to the results of Riccio and Matthews (1985) who report a 19% decrease in the number of symmetric synapses in the visual cortex contralateral to optic nerve impulse blockade by intraocular tetrodotoxin and to Winfield (1983) who reported a small reduction in the number of symmetric synapses in the visual cortex of kittens with eyelid suture. These data taken together with the fact that GABAergic terminals in the visual cortex form symmetric synapses (Ribak 1978; Somogyi and Hodgson 1985), indicate that blockade of nerve conduction and eyelid suture have a similar effect on cortical GABAergic synapses as enucleation. This effect appears to be a general one because Fifková (1970a) has reported a 20% reduction in the number of synapses in the neuropil (primarily the more common asymmetric synapses which are considered to be excitatory) of rat visual cortex following eyelid suture. Since the number of neurons does not change in the deprived visual cortex (Tsang 1937), these changes are probably due to a reduction in the development of the intracortical

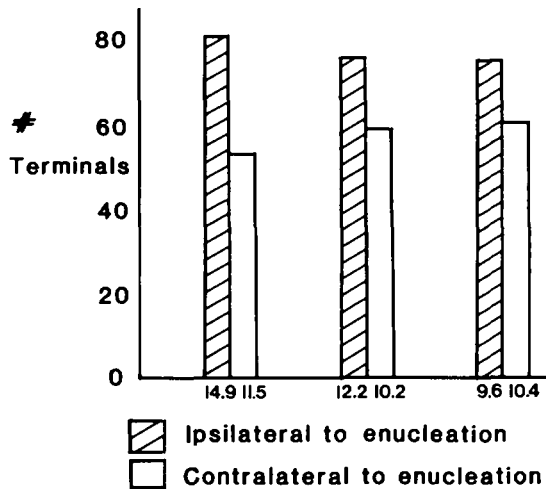


Fig. 2. Histogram that shows the number of GAD-positive puncta per 20,000  $\mu\text{m}^3$  in visual cortex for three rats with the ages of 18, 60 and 90 days, respectively. Samples were obtained at 100  $\mu\text{m}$  intervals from the pial surface down to the white matter. The numbers beneath each of the bars represent the standard deviations. Each of the differences in counts between the hemispheres is significant to the  $p < 0.01$  level

axonal plexuses. Sillito et al. (1981) have shown that eyelid suture also causes some physiological changes in the cat visual cortex that are consistent with the morphological changes observed for the GABAergic axon terminals in the present study.

These data are similar to the effects of neonatal deprivation in another sensory system. Benson et al. (1984) have shown in mice with closed nostrils that the number of mitral cells in the olfactory bulb is unaffected but the densities of excitatory and inhibitory synapses are reduced by 65% and 46%, respectively. Thus, it is apparent that sensory deprivation interferes with the formation or maturation of synapses.

These data along with other reports are suggestive of a functional balance between excitatory and inhibitory synapses, and presumably function, in the cortex. For example, Hendrickson et al. (1981) have shown that regions (puffs) of monkey visual cortex that show high levels of cytochrome oxidase activity also show greater numbers of GAD-containing neurons, suggesting that high metabolic activity is associated with greater numbers of GABAergic neurons. This correlation of metabolic activity and GABAergic neurons is supported by the present results which indicate that structures with reduced excitatory input have an associated decrease in inhibitory activity. Thus, a balance of excitatory and

inhibitory synaptic function appears to be maintained.

Recently, a similar type of study was made in the visual cortex of monocularly deprived kittens (Bear et al. 1985). The GAD-positive puncta appeared to remain uniform in layer IV even under conditions that produced alterations in cytochrome oxidase activity. However, quantitative differences between monocular segments of the two hemispheres were not analyzed and it remains unclear whether or not a small, but significant, loss of GABAergic terminals occurred. Although a biochemical analysis of GAD activity in the monocular and binocular segments did not demonstrate any consistent changes, it is possible that the levels of GAD in somata may have obscured the changes in the levels of GAD within axon terminals or that the specimens selected for analysis were not entirely from one segment or the other. Furthermore, it is clear that the visual cortex of kittens is more mature at the time of birth than that of rats. Morphological evidence indicates that geniculocortical axons enter the cortex of kittens by postnatal day 3 (Anker and Cragg 1974) and in the cortex of rats by postnatal day 6 (Mustari and Lund 1977). Similarly, visually evoked potentials are recordable in the visual cortex of kittens by day 4 (Rose and Lindsley 1968) but not in rats until day 8 (Rose and Ellingson 1970). These differences in the development of the visual cortex might explain why the rat displays a consistent loss of GABAergic terminals after neonatal monocular enucleation. Our data are supported by a recently published abstract (Hendry et al. 1985) that has reported a reduction in the number of GABA-positive puncta in visual cortex of monkeys following enucleation.

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## References

- Anker RL, Cragg BG (1974) Development of the extrinsic connections of the visual cortex in the cat. *J Comp Neurol* 154: 29-42
- Bear MF, Schmechel DE, Ebner FF (1985) Glutamic acid decarboxylase in the striate cortex of normal and monocularly deprived kittens. *J Neurosci* 5: 1262-1275
- Benson TE, Ryugo DK, Hinds JW (1984) Effects of sensory deprivation on the developing mouse olfactory system: a light and electron microscopic, morphometric analysis. *J Neurosci* 4: 638-653
- Creutzfeldt OD, Ito M (1968) Functional synaptic organization of primary visual cortex neurones in the cat. *Exp Brain Res* 6: 324-352

- Creutzfeldt OD, Kuhnt U, Benevento LA (1974) An intracellular analysis of visual cortical neurones to moving stimuli: responses in a co-operative neuronal network. *Exp Brain Res* 21: 251–274
- Fifková E (1970a) The effect of monocular deprivation on the synaptic contacts of the visual cortex. *J Neurobiol* 1: 285–294
- Fifková E (1970b) Changes of axosomatic synapses in the visual cortex of monocularly deprived rats. *J Neurobiol* 2: 61–71
- Hendrickson AE, Hunt SP, Wu J-Y (1981) Immunocytochemical localization of glutamic acid decarboxylase in monkey striate cortex. *Nature* 292: 605–607
- Hendry SHC, Jones EG, Kennedy MB (1985) Modulation of GABA, substance P and protein kinase immunoreactivities in monkey striate cortex following eye removal. *Soc Neurosci Abstr* 11: 16
- Lund RD, Mustari MJ (1977) Development of the geniculocortical pathway in rats. *J Comp Neurol* 173: 289–306
- Oertel WH, Schmechel DE, Mugnaini E, Tappaz ML, Kopin JJ (1981) Immunocytochemical localization of glutamate decarboxylase in rat cerebellum with a new antiserum. *Neuroscience* 6: 2715–2735
- Ribak CE (1978) Aspinous and sparsely-spinous stellate neurons in the visual cortex of rats contain glutamic acid decarboxylase. *J Neurocytol* 7: 461–478
- Ribak CE, Vaughn JE, Saito K (1978) Immunocytochemical localization of glutamic acid decarboxylase in neuronal somata following colchicine inhibition of axonal transport. *Brain Res* 140: 315–332
- Riccio RV, Matthews MA (1985) The postnatal development of the rat primary visual cortex during optic nerve impulse blockade by intraocular tetrodotoxin: a quantitative electron microscopic analysis. *Devel Brain Res* 20: 55–68
- Rose GH, Ellingson RJ (1970) Ontogenesis of evoked responses. In: Himwich WA (ed) *Developmental psychobiology*. Thomas CH, Springfield, pp 393–440
- Rose GH, Lindsley DB (1968) Development of visually evoked potentials in kittens: specific and nonspecific responses. *J Neurophysiol* 31: 607–623
- Sillito AM (1977) Inhibitory processes underlying the directional specificity of simple, complex and hypercomplex cells in the cat's visual cortex. *J Physiol* 271: 699–720
- Sillito AM, Kemp JA, Blakemore C (1981) The role of GABAergic inhibition in the cortical effects of monocular deprivation. *Nature* 291: 318–320
- Somogyi P, Hodgson AJ (1985) Antiserum to GABA: III. Demonstration of GABA in Golgi impregnated neurons and in conventional electron microscopic sections of cat striate cortex. *J Histochem Cytochem* 33: 249–257
- Tsang Y-C (1937) Visual centers in blinded rats. *J Comp Neurol* 66: 211–261
- Winfield DA (1983) The postnatal development of synapses in the different laminae of the visual cortex in the normal kitten and in kittens with eyelid suture. *Devel Brain Res* 9: 155–169

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